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Numerous metazoan cell types migrate extensively from their sites of birth to adopt positions critical for normal development. For example, in the vertebrate nervous system, neurons migrate to populate the peripheral nervous system. The objective of my research is to understand how migrating cells and neuronal growth cones are directed along their pathways. I conducted two screens for genes that are required for cell migration in the nematode *Caenorhabditis elegans*. One of the genes I identified, *cam-1*, specifies the final positions of migrating cells and orients cell polarity. *cam-1* appears to encode a receptor tyrosine kinase (RTK) most similar to the Ror subclass. Although other Ror proteins are known to be expressed in the developing nervous system, my analysis of *cam-1* mutants provides the first evidence that these proteins function in cell migration. In addition to *cam-1*, I identified six new genes, as well as six previously identified genes. Five of the previously identified genes encode proteins thought to be important for various aspects of cell migration in other organisms. This suggests that the new genes isolated in my screen are also likely to encode proteins that function directly in cell migration.

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FOREWORD

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8/27/97

Wayne Forrester 8/27/97

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INTRODUCTION

Numerous metazoan cell types migrate extensively from their sites of birth to adopt positions critical for normal development. For example, in many animals primordial germ cells move to the gonad during development. In the vertebrate central nervous system, neurons migrate radially from a proliferative zone to generate the layers of the cortex. Migrating neural crest cells populate the ganglia of the peripheral nervous system. Similarly, neuronal growth cones migrate to their synaptic targets, an essential step in achieving connectivity. The objective of my research is to understand how migrating cells and neuronal growth cones are directed along their pathways. In particular, I want to understand how migrating cells recognize and respond to extracellular guidance cues, and how their migrations are terminated at their proper destinations. I conducted two screens for genes that are required for cell migration in the nematode *Caenorhabditis elegans*. One of the genes I identified, *cam-1*, specifies the final positions of migrating cells and orients cell polarity. *cam-1* appears to encode a receptor tyrosine kinase (RTK) most similar to RTKs of the Ror subclass. Although other Ror proteins are known to be expressed in the developing nervous system (Masiakowski and Carroll, 1992; Oishi et al., 1997; Wilson et al., 1993), my analysis of *cam-1* mutants provides the first evidence that these proteins function in cell migration. In addition to *cam-1*, I identified seven new genes as well as previously identified genes. Five of the previously identified genes encode proteins thought to be important for various aspects of cell migration in other organisms. This suggests that the new genes isolated in my screen are also likely to encode interesting proteins that function directly in cell migration.

Cell migration is a complex process that requires many proteins. Extracellular matrix components and chemotropic agents function as cues that guide migrating cells along their proper pathways (Tessier-Lavigne and Goodman, 1996) and determine their final destinations (Thomas et al., 1990). Signals from extracellular guidance cues are transmitted by intracellular signaling molecules. Ultimately these signals converge to regulate cell polarity, cell adhesion and the actin cytoskeleton, which together generate the force that drives cell motility (Lauffenburger and Horwitz, 1996; Mitchison and Cramer, 1996).

Both biochemical and genetic approaches have been used to identify molecules involved in cell migration. I have chosen to use *C. elegans* as a model genetic system to study cell migration for several reasons. First, *C. elegans* is transparent so it is relatively simple to visually determine the positions of the cells that migrate. Additionally, *C. elegans* can easily be transformed to produce transgenic animals. Furthermore, the genomic sequence will be completed by 1998, which provides tools that facilitate the molecular cloning of gene identified by mutation. Finally, because many of the proteins required for cell migration are conserved in mammals, information learned about cell migration in *C. elegans* will be relevant to other organisms.

BODY

Experimental Methods, Assumptions And Procedures

Strains and genetics: Strains were grown at 20°C and maintained as described (Brenner, 1974). Strains bearing the following mutations were identified in the two genetic screens for cell migration mutants:
LGI: *unc-73(gm67)*, *unc-73(gm123)*, *cam-2(gm124)*, *syc-3(gm135)*
LGII: *cam-1(gm105)*, *cam-1(gm122)*
LGIII: *ceh-10(gm58)*, *ceh-10(gm71)*, *ceh-10(gm100)*, *ceh-10(gm120)*, *ceh-10(gm127)*, *ceh-10(gm131)*, *ceh-10(gm133)*, *syc-2(gm132)*, *fam-1(gm85)*, *ina-1(gm144)*, *syc-1(gm126)*
LGIV: *epi-1(gm57)*, *epi-1(gm121)*, *epi-1(gm139)*, *epi-1(gm146)*, *fam-2(gm94)*, *kyIs5* [*ceh-23-unc-76-gfp::lin-15*]
LGV: *unc-34(gm104)*, *unc-34(gm114)*, *unc-34(gm115)*, *unc-34(gm134)*, *vab-8(gm99)*, *vab-8(gm138)*
LGX: *mig-2(gm38)*, *mig-2(gm103sd)*, *kyIs4* [*ceh-23-unc-76-gfp::lin-15*]

The isolation and genetic characterization of Cam mutants *epi-1* (epithelialization defective), *ina-1* (integrin, α-subunit), *unc-34* (uncoordinated), *mig-2* (cell migration defective), *unc-73*, *vab-8* (variable abnormal), *ceh-10* (*C. elegans* homeobox), *cam-1*, *cam-2* (CAN abnormal migration), *syc-1*, *syc-2*, *syc-3*, (synthetic Cam), *fam-1* and *fam-2* (fasciculation and cell migration defective) are described by (Forrester et al.). The *ceh-10* alleles *gm71*, *gm100*, *gm131*, and *gm133* all appear identical to *gm58* for all phenotypes examined (Forrester et al.). *kyIs5* is a *ceh-23-gfp* reporter transgene that is integrated on LGIV, and *kyIs4* is the same reporter integrated on LGX (Zallen and Bargmann, personal communication).

Because most of the mutants were isolated in a strain containing the *kyIs5* reporter, we removed this transgene from the mutants by crossing to wild type. The *epi-1* alleles *gm121*, *gm139* and *gm146*, and the *fam-2(gm94)* allele are tightly linked to *kyIs5* and have not been separated from the reporter. For quantitative determinations of CAN axonal morphologies in mutants using green fluorescent protein (GFP; Chalfie et al., 1994), the *kyIs5* reporter was crossed into the mutant backgrounds. For *epi-1(gm57)*, which was isolated in a background lacking *kyIs5*, the *gm57* mutation was crossed into a *kyIs4* background.

Other than in *ceh-10(gm58)* mutants, all cell migrations were scored in homozygous mutant animals derived from homozygous parents. Because *ceh-10(gm58)* is lethal, we examined cell positions in homozygous animals derived from *ceh-10(gm58)/qC1* parents. Because *unc-73(gm67)* and *unc-73(gm123)* progeny of homozygous parents rarely survive to adulthood, we examined axonal morphologies of homozygous mutant animals derived from *unc-73/dpy-5(e61)* hermaphrodites. Similarly, because *epi-1(gm139)* animals are sterile, we examined axonal morphologies in mutant animals derived from *epi-1(gm139)/dpy-20(e1282*ts*) unc-30(e191)* hermaphrodites.

Scoring of axons and cells: Axons were scored as defective if they deviated from wild-type morphology. This morphology was defined by electron microscopic reconstructions of the *C. elegans* nervous system (White et al., 1986), immunocytochemical staining of wild-type animals (Desai et al., 1988; McIntire et al., 1992), and analysis of GFP expression in transgenic animals (Forrester and Garriga, 1997).

The extent of cell migration in wild-type and mutant animals was determined by comparing the positions of nuclei relative to coordinate non-migratory hypodermal nuclei using Nomarski optics. For the CANs, HSNs, ALMs, coelomocyte mother cells, Z1, Z4 and M mesoblast, cells that migrate embryonically, we scored in newly hatched hermaphrodite larvae the positions of the nuclei of these cells (or their progeny, the

coelomocytes, in the case of coelomocyte mother cells) relative to non-migratory hypodermal V and P nuclei. For the Q neuroblasts and their descendants, which migrate during the first larval stage, we scored in late first larval stage hermaphrodites the final positions of the Q descendant nuclei relative to the two daughter hypodermal nuclei derived from V1-6. GABA and serotonin expressing neurons were examined by indirect immunofluorescence as described (Forrester and Garriga, 1997).

Cloning *cam-1*: *cam-1* was mapped to a small interval using standard three-factor mapping (Brenner, 1974). Cosmids from this interval were used to generate transgenic animals, which were examined for rescue of the mutant phenotypes. Three overlapping cosmids were found to rescue *cam-1* mutant phenotypes. Subclones were generated from the cosmids and similarly assayed for their ability to rescue *cam-1*. A single 18 kilobase pair fragment of DNA was found to rescue. This fragment is predicted to contain a single gene using the genefinder sequence analysis program. We are currently sequencing the mutant *cam-1* alleles to verify that this gene is *cam-1*.

Results And Discussion

Identification of genes required for cell migration: We developed two screens for mutations that disrupted cell migration in *C. elegans*. They focused on a single representative cell type, the canal-associated neurons (CANs), which are a pair of bilaterally symmetric neurons that are born in the head and migrate to the middle of the animal during embryogenesis (Fig. 1). The CANs may function in osmoregulation and are essential for viability (Forrester and Garriga, 1997). By screening for mutants that displayed phenotypes associated with CAN defects and by screening for mutants with misplaced CANs, we identified 30 mutations that disrupted cell migration (Forrester et al.). These mutations defined 14 genes required for cell migration, half of which have not been identified previously. The screens identified alleles of the six previously defined genes *epi-1*, *ina-1*, *mig-2*, *unc-34*, *unc-73* and *vab-8*, and of the seven new genes *cam-1*, *cam-2* (CAN abnormal migration), *syc-1*, *syc-2*, *syc-3* (synthetic Cam), *fam-1* and *fam-2* (fasciculation and migration defective). The screens also identified the first mutations in the gene *ceh-10*, which encodes a homeodomain protein expressed in the CANs (Hawkins and McGhee, 1990; Svendsen and McGhee, 1995). With the exception of *mig-2(gm103sd)*, all of the mutations are recessive and therefore likely to reduce or eliminate gene function (Forrester et al.).

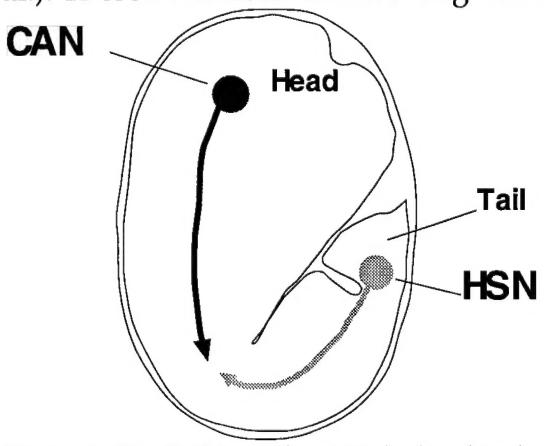

Figure 1. The CANs are born in the head and migrate to the middle of the animal during embryogenesis. The HSNs are born in the tail and migrate to a position just posterior to the CANs.

Although six genes had been described, our alleles of *mig-2* and *unc-73* behave differently from previously identified alleles of these genes. The *mig-2* allele *gm38* is recessive, whereas both the new allele *gm103* and the previously reported *mig-2* allele *rh17* are semidominant (Forrester et al.; Zipkin et al., 1997). Both *unc-73* alleles reported here lead to extensive lethality and sterility, phenotypes not produced by weaker *unc-73* alleles (Run et al., 1996).

Cell migration defects: To determine whether cell migrations other than those of the CANs were perturbed in the mutants, we examined the positions of several cells that

migrate during *C. elegans* development. We examined the positions of the ALM mechanosensory neurons, the HSN motor neurons, the mesodermal coelomocytes (ccs), the M mesoblast, and the Z1/Z4 somatic gonad precursor cells, all of which migrate during embryogenesis (Forrester and Garriga, 1997; Sulston et al., 1983). We also examined the positions of the Q neuroblast descendants and the P cell descendants. The left and right Q neuroblasts and their descendants migrate during the first larval stage (Sulston and Horvitz, 1977). The six bilaterally symmetric pairs of P cells cover the ventral third of newly hatched first larval stage (L1) animals. Midway through the L1, the P cells migrate ventrally and intercalate to form a row of twelve cells at the ventral midline. The cells then divide longitudinally to produce the Pn.a and Pn.p cells. The Pn.a neuroblasts divide during the L1 to produce ventral nerve cord motor neurons, and some of the Pn.p cells divide during the third larval stage to produce the hermaphrodite vulva.

Our analyses revealed that none of the mutations disrupted the migrations of Z1 and Z4, and only the *cam-2(gm124)* mutation appeared to disrupt M migration or development. We were unable to detect M in *cam-2(gm124)* mutants by Nomarski optics, suggesting that it was either severely misplaced or failed to differentiate normally.

Mutations in the nine genes *cam-1, epi-1, fam-1, fam-2, ina-1, mig-2, unc-34, unc-73* and *vab-8* affect the migrations of many cells in addition to the CANs (Forrester and Garriga, 1997). Mutations in the genes *epi-1, mig-2* and *unc-73* produce the most widespread defects, affecting all cell migrations scored except those of M, Z1 and Z4. *cam-1* mutations perturb the positioning of the CANs, ALMs, HSNs and ccsL, cells which normally migrate to positions near the middle of the animal (Forrester and Garriga, 1997).

Axonal outgrowth is disrupted in CAN migration mutants: Cell migration and axonal outgrowth are similar processes that require many of the same genes (for example, see (Hedgecock et al., 1987). To determine whether our mutations disrupt growth cone migrations, we examined CAN axonal morphology using the *ceh-23-gfp* reporter, HSN axonal morphology by anti-serotonin staining, and axonal morphology of the AVL, DVB, DD, and VD motor neurons by anti-GABA staining.

After the CAN cell bodies have migrated, they extend axons both anteriorly and posteriorly. To reach its normal destination in the tail, the posteriorly directed CAN axon from an anteriorly displaced CAN must extend further than the axon from a normally positioned CAN. Because posteriorly directed CAN axons in *ina-1(gm144)* and *mig-2(gm103*sd*)* mutants often extend to their normal destinations even when the cell bodies are in the head, CAN axonal defects observed in other Cam mutants probably reflect a direct requirement for gene function in CAN axonal outgrowth. Although all Cam mutations disrupted posteriorly directed CAN axonal outgrowth, the severity of such defects varied substantially. Mutations in *ceh-10* and *vab-8* severely disrupted CAN axonal outgrowth, mutations in *cam-1, cam-2, epi-1, unc-34,* and *unc-73* caused modest defects, and mutations in the remaining genes had little effect.

Each laterally positioned HSN cell body extends an axon ventrally along the epithelium to the ventral nerve cord, a paired bundle of axons that runs the length of the animal. Once in the cord, each axon turns anteriorly and extends along the ipsilateral axon bundle to the nerve ring, the major neuropil that encircles the pharynx. At the vulva, each HSN axon defasciculates from the ventral nerve cord and branches (Garriga et al., 1993; White et al., 1986). The mutants exhibited a range of HSN axonal outgrowth defects. In many mutants, the HSN cell bodies were displaced posteriorly,

consistent with results obtained by Nomarski optics. The HSN axons of *fam-2*, *mig-2*, *unc-34*, and *unc-73* mutants terminated prematurely in the ventral nerve cord before reaching the nerve ring, a phenotype previously described for *mig-2*, *unc-73*, and *unc-34* mutants (Desai et al., 1988; McIntire et al., 1992). The axons of normally positioned HSNs often failed to branch at the vulva in *epi-1*, *fam-2*, *ina-1* and *mig-2* mutants.

We also examined the axonal morphology of several GABAergic neurons. The DD motor neurons are generated during embryogenesis, and the VD motor neurons are generated during the L1. Both D-type motor neurons extend anteriorly directed processes along the ventral nerve cord that branch near their anterior ends. Each branch produces a commissure that extends dorsally along the lateral epithelium. Upon reaching the dorsal midline, the commissures branch and extend posteriorly and anteriorly directed processes along the dorsal nerve cord (McIntire et al., 1992). Dorsally directed commissural axons of *epi-1*, *fam-1*, *mig-2*, *unc-34*, and *unc-73* mutants often branched, stopped prematurely, or extended longitudinally along the lateral body wall, failing to reach the dorsal midline.

The ventral nerve cord often was disorganized in *epi-1*, *fam-1*, *fam-2*, *mig-2*, *unc-34*, and *unc-73* mutants. In wild-type animals, AVL and DVB also extend axons along the right ventral nerve cord (McIntire et al., 1992). Anti-GABA staining reveals that all four GABAergic axons extend near each other along the right bundle. The ventral nerve cords of twelve mutants representing six genes defasciculate, splitting into additional bundles that separate widely. This defasciculation was common in *epi-1*, *mig-2*, *unc-34*, and *unc-73* mutants and was occasionally seen in *fam-1* and *fam-2* mutants. Instead of extending as two separate axons, the HSNs of these mutants often extended both axons anteriorly as a single fascicle. This HSN defect and the GABAergic defasciculation defects seen in these mutants may result from general defects in ventral nerve cord fasciculation.

A gene that specifies cell position and polarity: Migrating cells must recognize when they have reached their proper destinations and stop migrating. One of the genes we identified, *cam-1*, appears to play a general role in this process. In *cam-1* mutants the positions of several migrating cell types are shifted anteriorly, suggesting that *cam-1* specifies the final position of multiple classes of cells.

In addition to recognizing spatial cues, the process of cell locomotion requires that a cell generate force in a single direction at its periphery. This is accomplished by the localized assembly of the protein complexes that generate the migratory force, possibly by regulating actin dynamics. Such polarization of cells is also seen in asymmetric cell division, where two daughter cells of a division differentially inherit the molecules that specify cell fate. *cam-1* also appears to play a role in polarizing cells. Six cells located laterally on each side of the animal, the V-cells, divide during the first larval stage to generate daughter cells that adopt different fates (Fig. 2). In *cam-1* mutants, the orientation of 10% of one of the V-cell divisions was reversed.

CAM-1 acts with a Wnt signaling protein to orient cell polarity: Multiple signaling pathways are likely to provide positional information along body axes to regulate cell position and polarity. For example, in *Drosophila* two secreted molecules, Wnt/Wg and DPP, act antagonistically to specify dorsal-ventral axis in developing imaginal disks (Theisen et al., 1996). Because of the similarities between *cam-1* and *egl-20* phenotypes, a strain mutant for both genes was generated. Analysis of the *cam-1; egl-20* double mutant revealed that the divisions of several V-cells now were reversed. These results suggest that *cam-1* encodes a signaling pathway component that acts with the EGL-20/Wnt

signaling pathway to direct cell migrations and orient asymmetric cell divisions along the A-P axis.

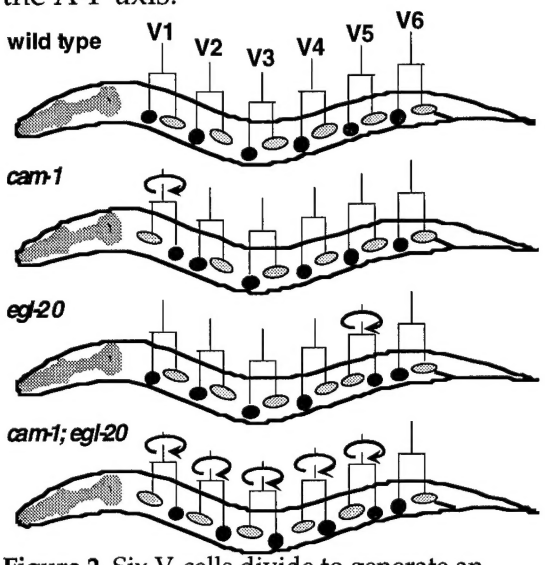

Figure 2. Six V-cells divide to generate an anterior cell that fuses with the syncitial epidermis (●) and a posterior blast cell (⬭).V1 polarity is reversed in *cam-1* mutants. V5 polarity is reversed in *egl-20* mutants. The polarity of V1 - V5 is reversed in *cam-1; egl-20* mutants.

***cam-1* appears to encode a receptor tyrosine kinase:** To further understand how *cam-1* acts to regulate cell position and polarity, I cloned the *cam-1* gene. *cam-1* appears to encode a RTK most similar to human and *Drosophila* Ror, proteins of unknown function that are expressed in neuronal tissues (Masiakowski and Carroll, 1992; Oishi et al., 1997; Wilson et al., 1993). The Ror proteins are closely related to the nerve growth factor receptor/Trk class of RTKs, and to MuSK, a protein involved in acetylcholine receptor clustering at the neuromuscular junction (Gillespie et al., 1996). Because RTKs can function as cell surface receptors for extracellular cues, one possibility is that CAM-1 functions directly to receive cues that specify cell position and cell polarity. The similarity to MuSK raises the possibility that Ror RTKs may act to assemble proteins that polarize cells. My future studies are aimed at understanding how Ror proteins function in cell migration.

Several genes function directly in cell migration: Mutations in eight genes perturb the migrations of many cells and neuronal growth cones. Pleiotropic defects in cell migration and axonal outgrowth caused by mutations in these genes suggest that they encode proteins that function directly in cell and growth cone migrations. Recent molecular analysis of four of these genes supports this view. *epi-1* encodes a laminin α chain homolog (Joh et al., personal communication). Laminin is a heterotrimeric extracellular matrix molecule that functions in cell and growth cone migrations (Lander, 1989). *ina-1* encodes an α integrin subunit (Baum and Garriga, 1997). Integrins are cell surface receptors that function in cell adhesion events necessary for cell and growth cone migrations (Hynes, 1992). *unc-73* encodes a putative regulator of Rho activity and *mig-2* encodes a Rho family member (Run et al., 1996; Zipkin et al., 1997). Rho family members function in fibroblast migration and attachment (Tapon and Hall, 1997). Therefore, four of the genes encode homologs to mammalian proteins implicated in cell migration. The four unidentified genes are likely to encode equally interesting proteins involved directly in cell migration.

Recommendations In Relation To The Statement Of Work

Below I list the five aims proposed in my Statement of Work, and discuss specific progress made towards achieving each aim:

Aim 1: Complete the analysis of the cell migration mutants identified in the screens to determine the precise number of genes identified (months 1-4).

Progress: This work was accomplished within the time frame proposed. The results are presented here (Experimental Methods, Assumptions and Procedures) and in two publications (Forrester and Garriga, 1997; Forrester et al.).

Aim 2: Complete phenotypic analysis of mutants to identify candidates most likely to be directly involved in cell migrations (months 1-12).
Progress: This work was completed within the time frame proposed. The results are summarized in this report and are presented in two publications (Forrester and Garriga, 1997; Forrester et al.).

Aim 3: Clone two of the genes already identified as likely to be directly involved in cell migrations (months 1-24).
Progress: One of the two genes was cloned within the first 12 months of the period of funding. Progress has been made towards the cloning of the second gene, *unc-34*. It should be pointed out that the naming of the genes has changed from the original proposal. The gene referred to as *cam-1* in the original proposal was defined by two alleles, *gm120* and *gm127*. Since the original proposal was submitted, we have discovered that these two alleles represented two unusual alleles of *ceh-10*, a *C. elegans* homeobox gene. We have presented the characterization of *ceh-10* mutations (Forrester et al.). We have named another gene, defined by two alleles, *gm105* and *gm122*, *cam-1*. Mutations in *cam-1* result in defects in multiple cell migrations as well as in cell polarity. To understand how *cam-1* acts to regulate cell position and polarity, we cloned the *cam-1* gene (Experimental Methods, Assumptions and Procedures). *cam-1* appears to encode a RTK most similar to human and *Drosophila* Ror (Results and Discussion).

Aim 4: Determine the phenotype in the absence of all gene function (months 6-18)
Progress: We have begun experiments to determine the phenotype of these mutants in the absence of all gene function. We have examined the phenotypes of *cam-1(gm105* and *gm122)* in trans to a chromosomal deficiency that deletes *cam-1*. The phenotypes of mutants bearing *cam-1(gm105)* are enhanced in trans to a chromosomal deficiency, suggesting that *cam-1(gm105)* does not remove all gene function. In contrast, mutants bearing *cam-1(gm122)* in trans to a chromosomal deficiency are no more severely defective than *cam-1(gm122)* mutants, suggesting that *cam-1(gm122)* may remove all gene function (Forrester et al.). To determine whether this is likely to be the case, we are determining the molecular nature of the lesion in *cam-1(gm122)*. The phenotypes of mutants bearing *unc-34* mutations in trans to deficiencies that delete the gene have been determined by our collaborators, and are no more severe than in the mutants alone, suggesting that *unc-34* mutations may be null.

Aim 5: Determine the sites of gene expression and function (months 12-24).
Progress: These experiments were proposed for the second year of funding. As a first step towards this goal, we have cloned one of the genes required for cell migration. The clone will be used to determine the site of gene expression using reporter transgene fusions and by antibody immunofluorescence. The site of gene function will be determined by mosaic analysis.

CONCLUSIONS

We have identified fourteen Cam genes that are required for *C. elegans* cell migrations. We find that these genes represent three classes: genes required for cell fate specification, genes required for multiple cell and growth cone migrations, and a single gene required for cell positioning .

Cam genes define three steps in cell migration: Analysis of the Cam mutants indicates that three genetically defined steps are necessary for cell migrations. First, cells decide to migrate. For the CANs, the gene *ceh-10,* either directly or through other genes, controls this decision to migrate. Second, cues guide the cell along its route. The gene *vab-8* guides cells and growth cones posteriorly (Manser and Wood, 1990; Wightman et al., 1996). Seven additional genes, *epi-1, fam-1, fam-2, ina-1, mig-2, unc-34,* and *unc-73,* function in multiple cell and growth cone migrations, and products of three of these genes function directly in cell and growth cone migrations as extracellular matrix molecules, cell surface receptors and signal transduction molecules. Third, extracellular cues define the final destinations of migrating cells. A cue from the CAN appears to stop the migrating HSN. In addition, the gene *cam-1* may function to define the final destinations of several migrating cells. Further molecular analysis of the genes that function in multiple cell migrations should lead to important insights into how the pathways and destinations of migrating cells are defined.

Eight genes function in multiple cell and growth cone migrations: Mutations in *epi-1, ina-1, fam-1, fam-2, mig-2, unc-34, unc-73,* and *vab-8* disrupt the migrations of multiple cells and growth cones. Cell migration and axonal outgrowth defects have been described previously for *mig-2, unc-34, unc-73* and *vab-8* (Desai et al., 1988; Hedgecock et al., 1987; Hedgecock et al., 1985; Manser and Wood, 1990; Wightman et al., 1996). The first *epi-1* and *ina-1* mutants were isolated on the basis of defects in epithelialization (E. Hedgecock, personal communication) and HSN migration (Baum and Garriga, 1997), respectively.

The *cam-1* gene functions in final positioning of migrating cells: In *cam-1(gm122)* mutants the HSNs migrate too far anteriorly 72% of the time. In addition to being excessive, HSN migrations in *cam-1* mutants can also be incomplete. Moreover, ALM migrations can also be incomplete or excessive in *cam-1* mutants, although the ALM defects are less severe than the HSN defects. In summary, cells appear to migrate to their approximate, but not precise, destinations in *cam-1* mutants. These results suggest that *cam-1* acts to define the final positions of several migrating cells.

Besides specifying final cell position, *cam-1* orients cell polarity. In *cam-1* mutants, the polarity of one of the V-cells is reversed some of the time. Interestingly, *cam-1* appears to orient cell polarity in concert with *egl-20,* which encodes a Wnt signaling molecule (Whangbo et al., personal communication). In animals mutant for both *cam-1* and *egl-20,* the polarities of additional cells are reversed.

We appear to have cloned the *cam-1* gene, and find that it is predicted to encode a receptor tyrosine kinase of the Ror family. Ror family members have been identified in Drosophila and mammals, where they are expressed in the developing nervous system (Masiakowski and Carroll, 1992; Oishi et al., 1997; Wilson et al., 1993). The function of Ror proteins is unknown. Analysis of *cam-1* will provide the first indication of Ror family member function.

REFERENCES

Baum, P., and Garriga, G. (1997). Neuronal migrations and axon fasciculation are disrupted in *ina-1* integrin mutants. Neuron *19*, 51-62.

Brenner, S. (1974). The genetics of Caenorhabditis elegans. Genetics *77*, 71-94.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W., and Prasher, D. C. (1994). Green fluorescent protein as a marker for gene expression. Science *263*, 802-5.

Desai, C., Garriga, G., McIntire, S. L., and Horvitz, H. R. (1988). A genetic pathway for the development of the Caenorhabditis elegans HSN motor neurons. Nature *336*, 638-46.

Forrester, W. C., and Garriga, G. (1997). Genes necessary for C. elegans cell and growth cone migrations. Develop *124*, 1831-43.

Forrester, W. C., Perens, E., Zallen, J. A., and Garriga, G. Identification of *C. elegans* genes required for neuronal differentiation and migration. Genetics accepted, pending revision.

Garriga, G., Desai, C., and Horvitz, H. R. (1993). Cell interactions control the direction of outgrowth, branching and fasciculation of the HSN axons of Caenorhabditis elegans. Develop. *117*, 1071-1087.

Gillespie, S. K., Balasubramanian, S., Fung, E. T., and Huganir, R. L. (1996). Rapsyn clusters and activates the synapse-specific receptor tyrosine kinase MuSK. Neuron *16*, 953-62.

Hawkins, N. C., and McGhee, J. D. (1990). Homeobox containing genes in the nematode Caenorhabditis elegans. Nucleic Acids Res *18*, 6101-6.

Hedgecock, E. M., Culotti, J. G., Hall, D. H., and Stern, B. D. (1987). Genetics of cell and axon migrations in Caenorhabditis elegans. Development *100*, 365-82.

Hedgecock, E. M., Culotti, J. G., Thomson, J. N., and Perkins, L. A. (1985). Axonal guidance mutants of Caenorhabditis elegans identified by filling sensory neurons with fluorescein dyes. Dev Biol *111*, 158-70.

Hynes, R. O. (1992). Integrins: versatility, modulation, and signaling in cell adhesion. Cell *69*, 11-25.

Joh, K., Hall, D., Yochem, J., Greenwald, I., and hedgecock, E., personal communication.

Lander, A. D. (1989). Understanding the molecules of neural cell contacts: emerging patterns of structure and function. Trends Neurosc. *12*, 189-195.

Lauffenburger, D. A., and Horwitz, A. F. (1996). Cell migration: a physically integrated molecular process. Cell *84*, 359-69.

Manser, J., and Wood, W. B. (1990). Mutations affecting embryonic cell migrations in Caenorhabditis elegans. Dev Genet *11*, 49-64.

Masiakowski, P., and Carroll, R. D. (1992). A novel family of cell surface receptors with tyrosine kinase-like domain. J Biol Chem *267*, 26181-90.

McIntire, S. L., Garriga, G., White, J., Jacobson, D., and Horvitz, H. R. (1992). Genes necessary for directed axonal elongation or fasciculation in C. elegans. Neuron *8*, 307-22.

Mitchison, T. J., and Cramer, L. P. (1996). Actin-based cell motility and cell locomotion. Cell *84*, 371-9.

Oishi, I., Sugiyama, S., Liu, Z. J., Yamamura, H., Nishida, Y., and Minami, Y. (1997). A novel Drosophila receptor tyrosine kinase expressed specifically in the nervous system. Unique structural features and implication in developmental signaling. J Biol Chem *272*, 11916-23.

Run, J.-Q., Steven, R., Hung, M.-S., Van Weeghel, R., Culotti, J. G., and Way, J. C. (1996). Suppressors of the unc-73 gene of Caenorhabditis elegans. Genetics *143*, 225-236.

Sulston, J. E., and Horvitz, H. R. (1977). Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. Dev Biol *56*, 110-56.

Sulston, J. E., Schierenberg, E., White, J. G., and Thomson, J. N. (1983). The embryonic cell lineage of the nematode Caenorhabditis elegans. Dev. Biol. *100*, 64-119.

Svendsen, P. C., and McGhee, J. D. (1995). The C. elegans neuronally expressed homeobox gene ceh-10 is closely related to genes expressed in the vertebrate eye. Develop.

Tapon, N., and Hall, A. (1997). Rho, Rac and Cdc42 GTPases regulate the organization of the actin cytoskeleton. Curr Opin Cell Biol *9*, 86-92.

Tessier-Lavigne, M., and Goodman, C. S. (1996). The molecular biology of axon guidance. Science *274*, 1123-33.

Theisen, H., Haerry, T. E., O'Connor, M. B., and Marsh, J. L. (1996). Developmental territories created by mutual antagonism between Wingless and Decapentaplegic. Development *122*, 3939-48.

Thomas, J. H., Stern, M. J., and Horvitz, H. R. (1990). Cell interactions coordinate the development of the C. elegans egg-laying system. Cell *62*, 1041-52.

Whangbo, J., Harris, J., Hongeward, G., and Kenyon, C., personal communication.

White, J. G., Southgate, E., Thomson, J. N., and Brenner, S. (1986). The structure of the nervous system of Caenorhabditis elegans. Philos. Trans. R. Soc. Lond. B Biol. Sci. *314*, 1-340.

Wightman, B., Clark, S. G., Taskar, A. M., Forrester, W. C., Maricq, A. V., Bargmann, C. I., and Garriga, G. (1996). The C. elegans gene vab-8 guides posteriorly directed axon outgrowth and cell migration. Development *122*, 671-82.

Wilson, C., Goberdhan, D. C., and Steller, H. (1993). Dror, a potential neurotrophic receptor gene, encodes a Drosophila homolog of the vertebrate Ror family of Trk-related receptor tyrosine kinases. Proc Natl Acad Sci U S A *90*, 7109-13.

Zipkin, I. D., Kindt, R. M., and Kenyon, C. J. (1997). Role of a new Rho family member in cell migration and axon guidance in *C. elegans*. Cell *in press*.